# Crystal Structure of the DNA-binding Domain of the Replication Initiation Protein E1 from Papillomavirus

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Beamline(s): **X8C, X26C**

**Introduction**: Papillomaviral infection causes both benign and malignant lesions and is a necessary cause of cervical carcinoma. Replication of this virus requires the replication initiation proteins E1 and E2, which bind cooperatively at the origin of replication (*ori*) as an $(E1)_2$-$(E2)_2$-DNA complex. This complex is a precursor to larger E1 complexes that distort and unwind the *ori*.

**Methods and Materials**: We determined the crystal structure of the E1 DNA-binding domain (E1-DBD) by using a three-wavelength data set for a Se-Met derivative crystal collected at beamline X8C and a single wavelength data set for a native crystal collected at beamline X26C. The structure was solved by using Multiple Anomalous Dispersion (MAD) after determination of eight Se sites by the program SnB (Weeks and Miller, 1999) and refinement of these by the program SHARP (de La Fortelle and Bricongne, 1997). Subsequently, several bromide atoms were located by Fourier synthesis in the native crystal, which improved the phase model by isomorphous difference with the Se-Met derivative crystal (crystallized from chloride). Solvent-flattening and NCS-averaging led to a fully traceable electron density map. The model was built with the program O (Jones *et al.,* 1991) and refined to 1.9 Å resolution with the program CNS (Brünger *et al.* 1998) to an Rcryst of 0.242 and Rfree of 0.272.

**Results and Conclusions**: The E1-DBD structure consists of a central five-stranded antiparallel $\beta$ sheet flanked by loosely packed $\alpha$ helices on one side and more tightly packed helices on the other. Residues critical for DNA binding are located on an extended loop and an $\alpha$-helix. Although the extended DNA-binding loop does not possess secondary structure, it is well defined in the 3D structure. Selective mutations at an E1/E1 interface observed in the crystal structure identified the E1:E1 dimerization surface within the $(E1)_2$-DNA complex, and allowed the proposal of a model for such a complex (**Figure 1**). These and other observations suggest how the E1 DNA-binding domain orchestrates assembly of the hexameric helicase on the *ori*. For further details see Enemark et al.

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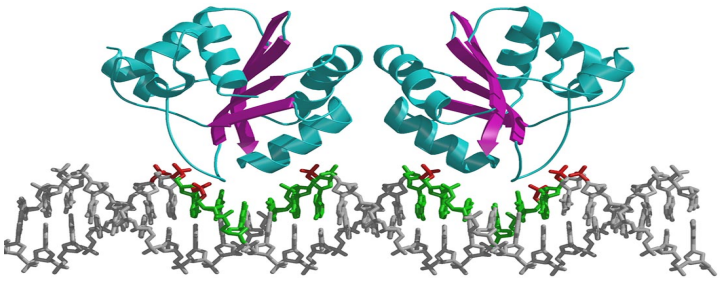

**Figure 1**. A crystallographic interaction between E1 monomers reveals a potential model for E1/E1 interaction in which the distance between the two DNA binding surfaces corresponds to the separation between two major grooves of DNA. The resulting E1 dimer was docked onto a DNA double helix containing the *ori* sequence and the model was tested by mutagenesis (see text).